PROCESS FOR PURIFYING PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 10/895,581, filed Jul. 21, 2004, now allowed, which claims the benefit of U.S. provisional application No. 60/540,587, filed Jan. 30, 2004, the entire disclosure of which is relied on and incorporated by reference.

FIELD OF THE INVENTION

This invention relates to protein purification and specifically to a process for protein purification using hydrophobic interaction chromatography.

BACKGROUND OF THE INVENTION

The purification of proteins for the production of biological or pharmaceutical products from various source materials involves a number of procedures. Therapeutic proteins may be obtained from plasma or tissue extracts, for example, or may be produced by cell cultures using eukaryotic or procaryotic cells containing at least one recombinant plasmid encoding the desired protein. The engineered proteins are then either secreted into the surrounding media or into the perinuclear space, or made intracellularly and extracted from the cells. A number of well-known technologies are utilized for purifying desired proteins from their source material. Purification processes include procedures in which the protein of interest is separated from the source materials on the basis of solubility, ionic charge, molecular size, adsorption properties, and specific binding to other molecules. The procedures include gel filtration chromatography, ion-exchange chromatography, affinity chromatography, and hydrophobic interaction chromatography.

Hydrophobic interaction chromatography (HIC) is used to separate proteins on the basis of hydrophobic interactions between the hydrophobic moieties of the protein and insoluble, immobilized hydrophobic groups on the matrix. Generally, the protein preparation in a high salt buffer is loaded on the HIC column. The salt in the buffer interacts with water molecules to reduce the solvation of the proteins in solution, thereby exposing hydrophobic regions in the protein which are then adsorbed by hydrophobic groups on the matrix. The more hydrophobic the molecule, the less salt is needed to promote binding. Usually, a decreasing salt gradient is used to elute proteins from a column. As the ionic strength decreases, the exposure of the hydrophilic regions of the protein increases and proteins elute from the column in order of increasing hydrophobicity. See, for example, *Protein Purification,* 2d Ed., Springer-Verlag, New York, 176-179 (1988).

When developing processes for commercial production of therapeutically important proteins, increasing the efficiency of any intermediate purification steps is highly desirable. One way of improving the ease and efficiency of manufacturing is to increase the load capacity of one or more of the intermediate steps of the purification process to the point that the number of cycles required to purify a batch of protein is reduced without compromising the quality of the protein separation. The present invention improves the process of protein purification by increasing the capacity and efficiency of an intermediate step.

SUMMARY OF THE INVENTION

The present invention provides a process of purifying a protein comprising mixing a protein preparation with a solution containing a first salt and a second salt, forming a mixture which is loaded onto a hydrophobic interaction chromatography column, wherein the first and second salts have different lyotropic values, and at least one salt has a buffering capacity at a pH at which the protein is stable. In one embodiment, the pH of the mixture and equilibrium buffer is between about pH 5 and about pH 7. The process further comprises eluting the protein.

The present invention provides combinations of salts useful for increasing the dynamic capacity of an HIC column compared with the dynamic capacity of the column using separate salts alone. These combinations of salts allow for a decreased concentration of at least one of the salts to achieve a greater dynamic capacity, without compromising the quality of the protein separation. The first and second salt combinations are selected for each particular protein through a process of establishing precipitation curves for each salt individually, and precipitation curves for the combination of salts holding one salt constant and varying the second. The concentrations of the salt combinations can be optimized further, for example, to ensure protein stability at room temperature and to prevent formation of aggregates in the protein preparation.

Preferred first salts are those which form effective buffers at a pH at which the protein is stable. In one embodiment, the first and second salts are selected from acetate, citrate, phosphate, sulfate, or any mineral or organic acid salt thereof. In one embodiment the pH of the mixture is between about pH 5 and about pH 7. In one embodiment, the final salt concentrations of the first salt and second salts in the mixture are each between about 0.1 M and 1.0 M, in another embodiment between about 0.3 M and about 0.7 M. The cations can be selected from any non-toxic cations, including NH_4^+, K^+, and Na^+. Preferred cations are those which do not tend to denature the protein or to cause precipitation in combination with other ions, including NH_4^+ and Na^+.

The two salt buffers of the present invention result in an increase in dynamic capacity of an HIC column for a particular protein compared with the dynamic capacity achieved by single salts. This results in decreased number of cycles required for purifying a batch of protein. Therefore, the present invention has special applicability to commercial manufacturing practices for making and purifying commercially important proteins.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows dual salt precipitation curves for an antibody against EGFR performed as described in Example I below. FIG. **1**A shows the precipitation curve for 0.5 M sodium sulfate with increasing concentrations of sodium phosphate and the precipitation curve for 0.4 M sodium phosphate with increasing concentrations of sodium sulfate. FIG. **1**B shows the precipitation curves for 0.55 M sodium citrate with increasing concentrations of sodium phosphate, and 0.4 M sodium phosphate with increasing concentrations of sodium citrate. FIG. **1**C shows the precipitation curves for 0.6 M sodium acetate with increasing concentrations of sodium sulfate, and 0.5 M sodium phosphate with increasing concentrations of sodium sulfate. FIG. **1**D shows the precipitation curves for 0.6 M sodium acetate with increasing concentrations of sodium citrate, and 0.55 M sodium citrate with increasing concentrations of sodium acetate. FIG. **1**E shows the precipitation curves for 0.55 M sodium citrate with